I. L. KOSIN

A STUDY OF THE MORPHOLOGY OF MORIBUND TURKEY BLASTODERMS

by

I. L. Kosin

WASHINGTON AGRICULTURAL EXPERIMENT STATIONS

####

Reprinted from "Official Reports, 9th World's Poultry Congress"
Section III, Vol. 3, pp. 75-87
Paris, August 3-9, 1951

A STUDY OF THE MORPHOLOGY OF MORIBUND TURKEY BLASTODERMS[1,2]

I. L. Kosin

Department of Poultry Husbandry
The State College of Washington
Pullman

Review of Literature:

The matter of gross macroscopic differences between "fertile" and "infertile" germ discs of chicken eggs has long been a subject of considerable interest both to the general embryologist and to the investigator interested in the more applied aspects of avian embryonic development. His (1868), Oellacher (1872), Barfurth (1895), and Lécaillon (1910), as well as others, have made important fundamental contributions in this field. The more recent observations of Hays and Nicolaides (1934), Kosin (1944, 1945), and Gowe (1950) helped to lay a firm foundation for the use of these differences in hatchability investigations.

Three years ago, the writer briefly referred to the fact that the turkey germ disc closely approximates in its macroscopic appearance that of the chicken disc (Kosin, 1948). Since then, considerable data have been accumulating at this laboratory pertaining to turkey germ disc classification and early embryonic mortality. The degree of prevalence of the latter in the Broad Breasted Bronze breed of turkeys is being reported elsewhere (Kosin, 1951). The present report is primarily concerned with describing gross and microscopic appearance of moribund (and dead?) turkey blastoderms. The existence of such discs in chicken eggs apparently was first suspected by Barfurth (1895). He was inclined to believe that such blastoderms are the product of syngamy between the normal ova and the "overripe" spermatozoa. Taylor and Gunns (1939) attempted to discover a relationship between certain linear measurements of the chicken blastoderm and hatchability. Their results were largely negative.

The possibility that the developmental stage of the egg at the time of ovi-position may have a bearing on its hatchability potential was suggested by Stockard (1921) and studied at some length by Hays and Nicolaides (1934). The writer briefly discussed the matter of moribund and dead turkey blastoderms as a factor in hatchability in an abstract in 1949 (Kosin, 1949). Gowe (1950) has discussed the appearance of "degenerate" chicken egg blastoderms which, he suggested, are incapable of normal development.

---

[1] Published as Scientific Paper No. 991, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, The State College of Washington, Pullman.

[2] The study reported here was supported by funds provided under the Research and Marketing Act, of 1946 and conducted at The Washington Agricultural Experiment Station as a part of the Western Regional Poultry Breeding Project.

Studies at this laboratory (Kosin, 1951) showed that about 20 per cent of the incubated Broad Breasted Bronze hatching eggs, classified as "infertiles" by candling, on breaking showed definite signs of early arrested development. This led the writer to suspect the existence of morphongenetically moribund turkey blastoderms which may be detectable by a macroscopic examination. Accordingly a systematic study was undertaken aimed at correlating the gross macroscopic appearance of the turkey blastoderm with its development during the first 5 days of incubation. The objective was to identify, if possible, the blastoderms which could account for the above-mentioned 20% candling error.

Materials and Methods:

The procedure was as follows. The Broad Breasted Bronze hatching eggs were set on the small end overnight in order to have the germ disc floating on the side facing the upper (large) end of the egg. Next morning, a circular opening about 25 mm. in diameter was cut around the blunt end axis, and the shell membranes were carefully removed in the area. The exposed germ disc was then classified into one of three groups: (1) fertile, (2) infertile, or (3) doubtful. Because this report is not concerned with group (2) eggs, no further mention will be made of them.

Some of group (3) eggs were subjected to the rapid alum cochineal staining method (Kosin, 1944) to assure a correct classification. The bulk of these were found to be fertile, although even such relatively superficial examination usually revealed that the cell distribution over the top of these blastoderms was more irregular and spotty than that found in group (1) eggs. Because it was considered important to study the development of group (3) eggs, representative specimens were chosen at random to be further studied with group (1) eggs.

The latter, together with the just-mentioned group (3) blastoderms, were handled in the following manner. A freehand sketch was made of each blastoderm with the aid of 12x wide angle stereoscopic microscope. The use of blue filter with the reflected type of illumination was found helpful in bringing the whole blastoderm into a bolder relief. The sketch included all of the essential landmarks and peculiarities of a blastoderm. The circular opening was then covered with a small watch glass, sealed with paraffin, and the egg placed standing on its small end in an isothermic oven maintained at 38°C. Additional humidity was provided. Needless to say, the above operations were performed under reasonably asceptic conditions: before cutting through the shell the involved area was swabbed with 70% ethanol; all instruments and watch glasses were sterilized.

After the eggs were placed into the oven, they were examined through the window at least once a day to check on the progress or lack of embryonic development. If the egg appeared to be developing normally on the fifth day, it was removed, as such a blastoderm was of no further interest insofar as the present study was concerned. If, on the other hand, the development was erratic, slow, teratological, or appeared to have come to a standstill during this period, then additional drawings were made to illustrate these conditions. The method of in-situ cultivation of embryos, as adopted in this study, appeared to have, for the purposes of the investigation, certain obvious advantages over the in-vitro method developed by Romanoff (1943). In a number of cases, histological preparations were made. Where possible, photomicrography was used to augment the drawings. In all, 288 blastoderms were studied this way.

Results and Conclusions:

Figs. 1 and 2 show a normal unincubated turkey blastoderm, while the infertile germ disc is illustrated in figs. 3 and 4. The former is characterized by two landmarks: the semi-translucent central area pellucida and the more solid peripheral area opaca. The nucleus of Pander as a rule is visible as a white mass underlying the central portion of area pellucida. The presence of this white yolk mass makes the correct identification of the infertile turkey eggs somewhat more difficult than that of the fowl. In the latter, the nucleus of Pander is less pronounced in the fertile egg. The significance of this will be fully appreciated if it is remembered that the main distinguishing characteristic between "infertile" and "fertile" germ discs in the presence of a relatively extensive area pellucida in the latter and its absence in the blastodisc. The presence of a large white yolk mass under the area pellucida may obscure it sufficiently to cause an incorrect classification.

As will be shown, the presence or absence of lacunae in itself does not differentiate between a fertile and an infertile germ disc. The author considers a normal turkey blastoderm to be a "fertile" germ disc which has no lacunae in the area pellucida and only a few on the outer periphery. The absence of the area pellucida and the presence of more or less numerous lacunae both on the periphery and in the central portion of the disc characterizes an infertile egg (figs. 3 & 4). The lacunae (or vacuoles, as they were termed by Lécaillon, Barfurth, and their predecessors) vary in size, shape, and distribution, and usually are absent from the central portion of the blastodisc's surface. In cross section, the lacunae appear to be fluid-filled, and acidophilic in their staining reaction. The fixed preparations (fig. 5) often show debris inside of the lacuna, probably the precipitated proteins of the fluid.

While the above descriptions fit the "model" fertile and infertile germ discs, deviations from these models have been observed. For example, in the blastodiscs, the lacunae may appear as a system of interconnected canals (fig. 6). In this type, the central portion appears solid white.

In normal blastoderms, the variations are largely limited to the relative size of the component parts reflecting differences in the developmental stages of individual blastoderms caused by clutch size and time of oviposition.

The more extensive deviations from normality almost invariably indicate the state of moribundity or even death.

A blastoderm type which, on incubation, had invariably failed to develop beyond the first few hours is illustrated in figs. 7 and 8. At oviposition, such blastoderms are small in diameter, seldom measuring more than 3.5 mm. The area pellucida is not apparent in a superficial examination. The margin of overgrowth is "ragged." Following a 24-hour incubation, the blastoderm usually doubles its area, though still retaining its essentially undifferentiated appearance. The development never proceeds to the blood islet stage. An histological examination of an unincubated blastoderm of this type reveals a one or two cell thick layer of tissue distributed rather evenly over the entire embryonic area. Essentially, the growth pattern of these blastoderms resembles that of an actively proliferating tissue culture. It has much in common with Anidian blastoderms found in sauropsid eggs. Anidians are characterized by active cell proliferation and a complete absence of axiation and differentiation (Needham, 1942).

A common type of moribund blastoderm is illustrated in fig. 9, where signs of cytoplasmic degeneration are in evidence around its entire periphery. In all other respects, the disc appears normal. A histological examination of such discs reveals an essentially normal pattern of embryonic growth except for the tissue involution on the disc's periphery. The development ceases within 36 hours. In extreme cases, such embryos do not develop beyond the early primitive streak stage (figs. 10 and 11). Whether the type illustrated in fig. 12 is an earlier version of the degenerative process shown in figs. 9 and 10 is not known. But again, the margin of overgrowth is seen to be eroded in one region. The embryo survived for 3 days but did not develop beyond the 24-30 hour stage. Moreover, the eroded area was still visible at the time the embryo died (fig. 13).

It has been mentioned earlier that the presence of numerous lacunae on the periphery (area opaca) does not necessarily indicate that the egg is infertile. Thus a type of the moribund blastoderm is shown in fig. 1 in which the apparently normal are pellucida is surrounded by an area opaca pock-marked with lacunae. When incubated, such blastoderms do not increase in diameter - all the growth is centripetal, as if tangential growth is precluded by the eroded area opaca (fig. 15). When examined in more detail (fig. 16), the embryonic mass presents a contrasting picture of the solidly packed cells with small, round nuclei, and the more amorphous lower layer where clusters of cells can be seen sloughing from the underside of the top layer. Everything points to active cellular proliferation.

A far less common type of the moribund blastoderm is shown in fig. 17 in which a slight erosion of the margin of overgrowth is accompanied by the presence of an extra large area pellucida. The latter appears to be imperceptibly divided into two sections by the underlying white yolk mass which is split in two. On incubation, one of the area pellucida segments gives rise to an apparently normal embryo which survives for 3-4 days, while the other segment resolves itself into an area devoid of any traces of true embryonic development (fig. 18). Histologically, the latter appears merely as a thin layer of undifferentiated cells. The surrounding blood islets of the "dominant" segment do not invade the undifferentiated area. Here one is tempted to speculate that this type of blastoderm is an example of a conflict between two competing organizers.

The types of abnormal blastoderms described in the preceding sections of this paper by no means cover the entire range of gross morphological variations which had been observed. They merely represent the more frequently encountered types which, it is believed, are in a sense responsible for the bulk of the early postovipostial blastoderm mortality. When all of these types are considered, then it seems their occurrence is frequent enough to account for at least a large proportion of the error in candling.

The fact that these moribund blastoderms (some of them in reality dead) can be detected by a visual examination is not surprising. Observations at this laboratory show that, in an infertile egg, the lacunae develop rapidly while the egg is still in the uterus. They are not to be found in the blastodiscs of eggs in the magnum or isthmus (Lécaillon, 1910). This suggests that their presence is a certain sign of degeneration of varying degrees of intensity. Oellacher (1872) reported that he had never observed vacuoles(lacunae) in the in-utero fertile chicken eggs, a point which has been corroborated at this laboratory both on chicken and turkey eggs. In those fertile eggs in which these lacunae are to appear, the latter show up sometime during the first

few hours after oviposition. Depending on the degree of degeneration, the development of these lacunae may be of a limited nature of continue progressively until the blastoderm dies. The latter fact undoubtedly, in a large measure, accounts for the multiplicity of types of moribund turkey blastoderms observed in the present investigation.

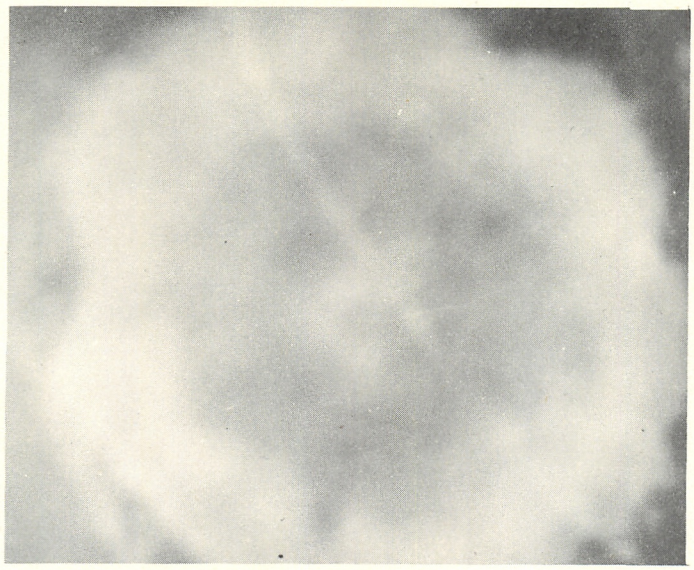


Fig. 1. A photomicrograph of a normal turkey blastoderm. Note the white yolk mass in the center showing through the relatively translucent area pellucida. The lacunae like structures on the periphery are artifacts. The line running across the face of the blastoderm was caused by a fold in the vitelline membrane. x30.

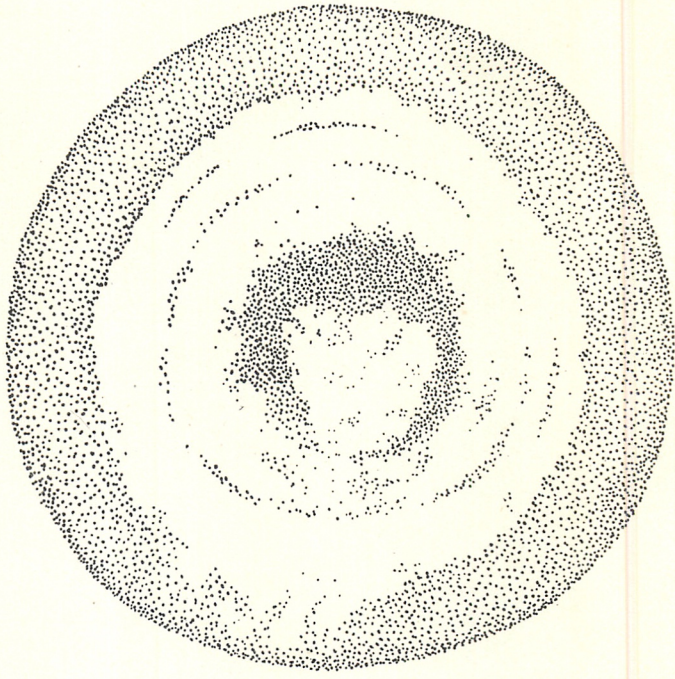
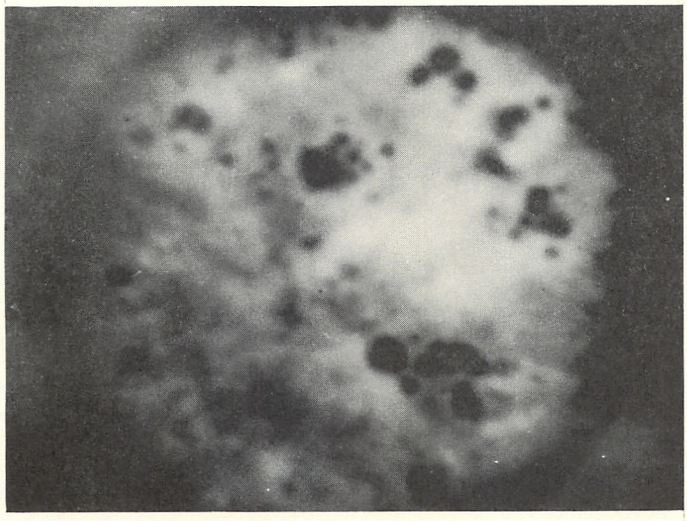
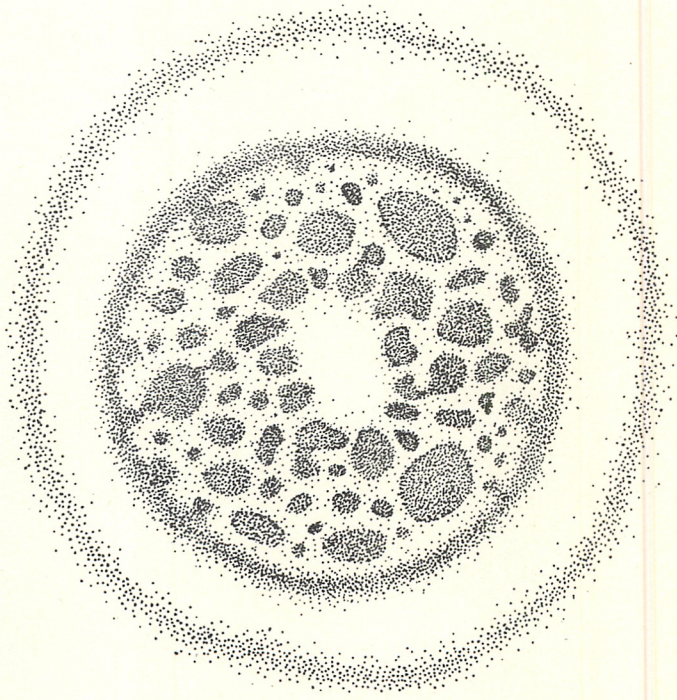


Fig. 2. A drawing of a normal turkey blastoderm. The stippled area in the center represents the area pellucida with the underlying yellow yolk showing through as dark mass. The nucleus of Pander is shown as a white mass just slightly off center. x15.





Figs. 3 and 4. A photomicrograph (x30) and a drawing (x15), respectively, of a typical infertile turkey germ disc. Note the numerous lacunae, and a solid white area, free of lacunae, near the blastodisc's center. The photomicrograph does not show the outer periblastic ring which, however, is indicated in the drawing.

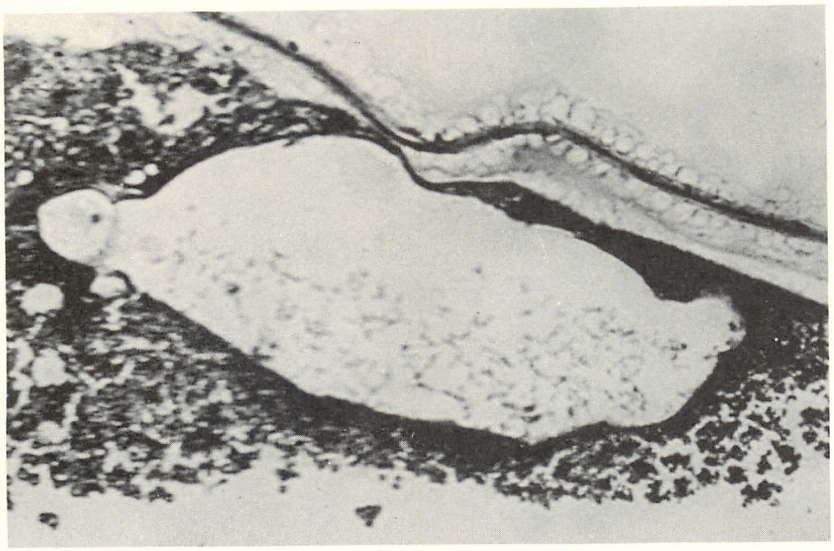


Fig. 5. A lacuna in crossection. Note the debris. x200.

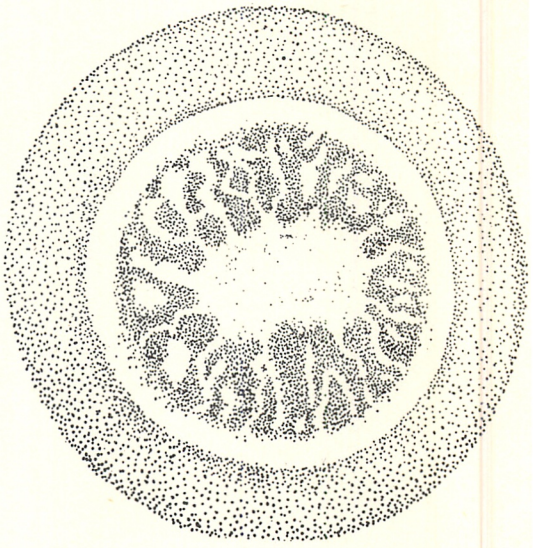


Fig. 6. A variant in turkey blastodisc. x10.

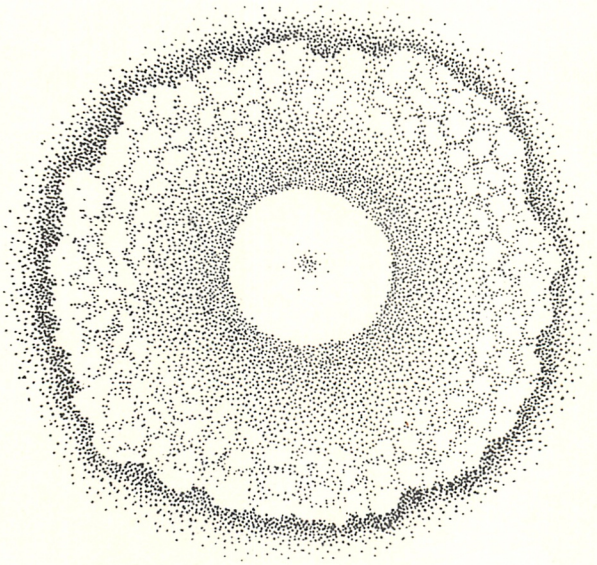


Fig. 7. A variant of moribund blastoderm. Note its small overall size (and a correspondingly insignificant area pellucida?). x10.

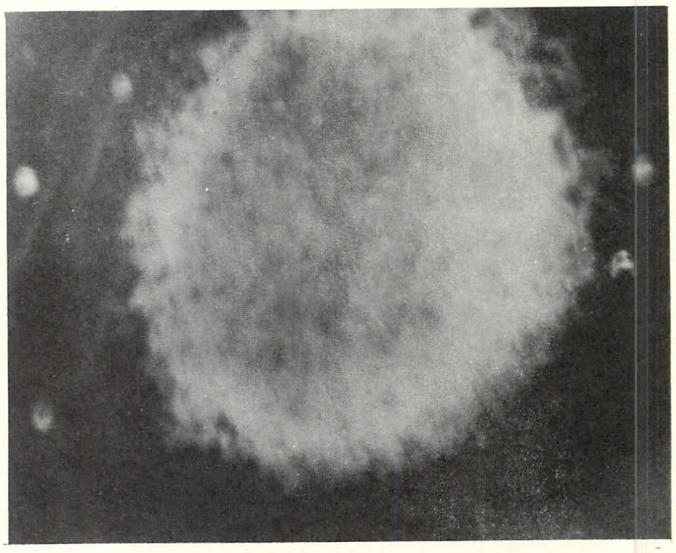


Fig. 8. A photomicrograph of the same type of moribund blastoderm illustrated in fig. 7. Note the apparent absence of the area pellucida, and the ragged edges of the embryonic growth area (margin of overgrowth?). x35.

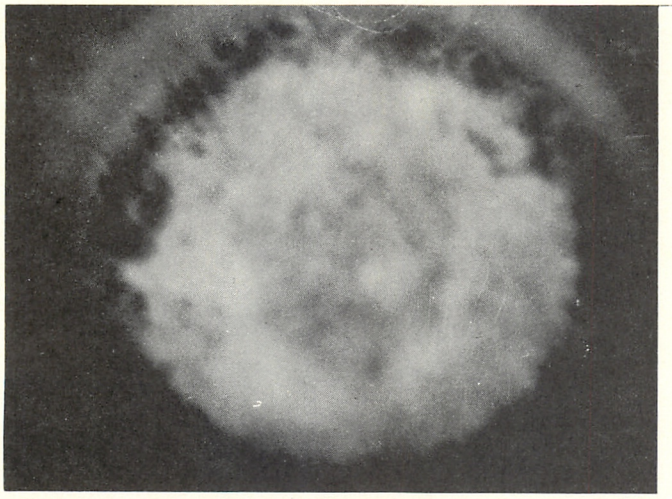
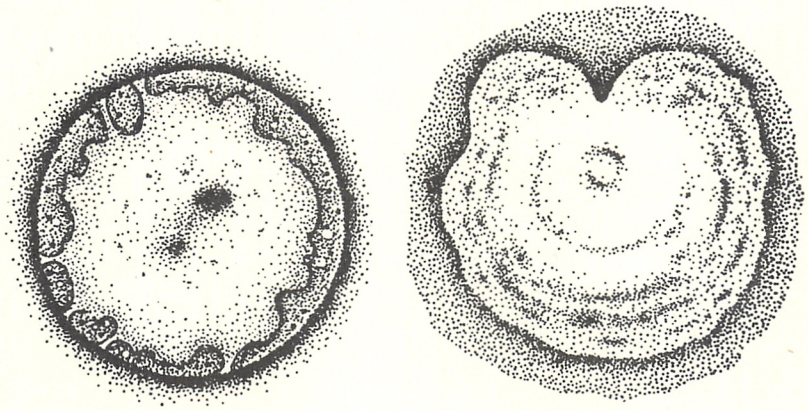
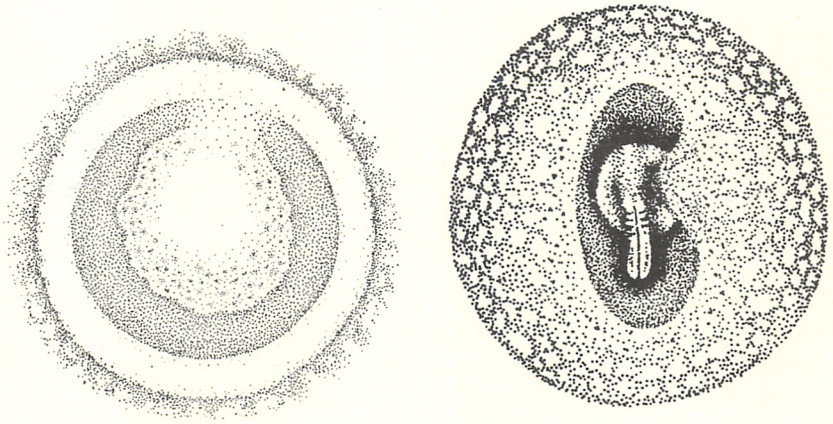


Fig. 9. A photomicrograph of another variant of moribund turkey blastoderm. Note extreme tissue degeneration along the margin of overgrowth. x30.



Figs. 10 and 11. Drawings of the variant, illustrated in fig. 9, before and after 5 days incubation. Development ceased at an early primitive streak stage. x12.



Figs. 12 and 13. Drawings of a variant of moribund turkey blastoderm before and after 5 days incubation. An "erosion" of the area pellucida, visible in the unincubated blastoderm (fig. 12. x12) is retained by the developing embryo (fig. 13. x6).

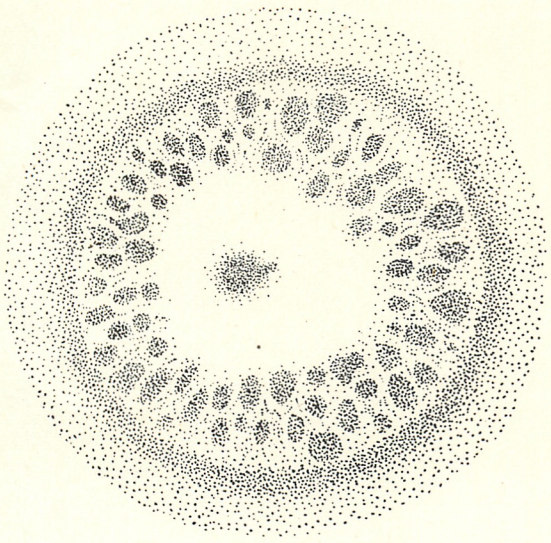


Fig. 14. A variant of moribund turkey blastoderm. Note the large area pellucida and the "pock-marked" area opaca. x20.

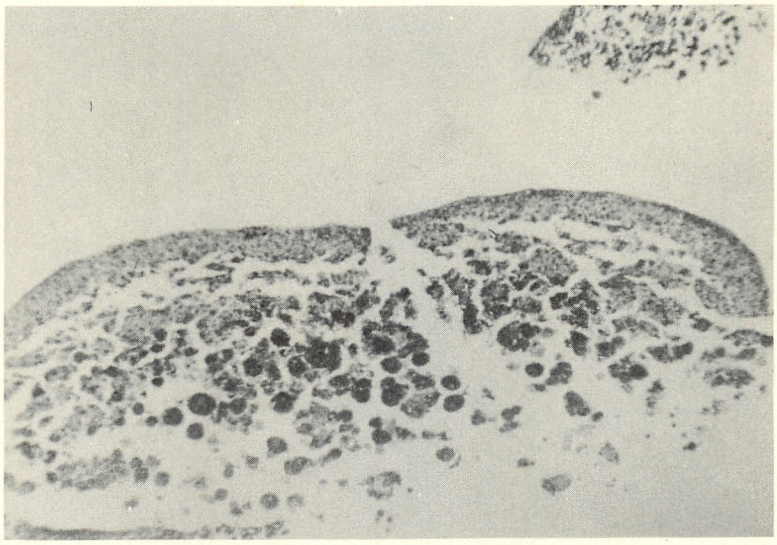


Fig. 15. A section of the blastoderm shown in fig. 14. The break in the center is an artifact. Note the sharply delimited area of embryonic growth. x30.

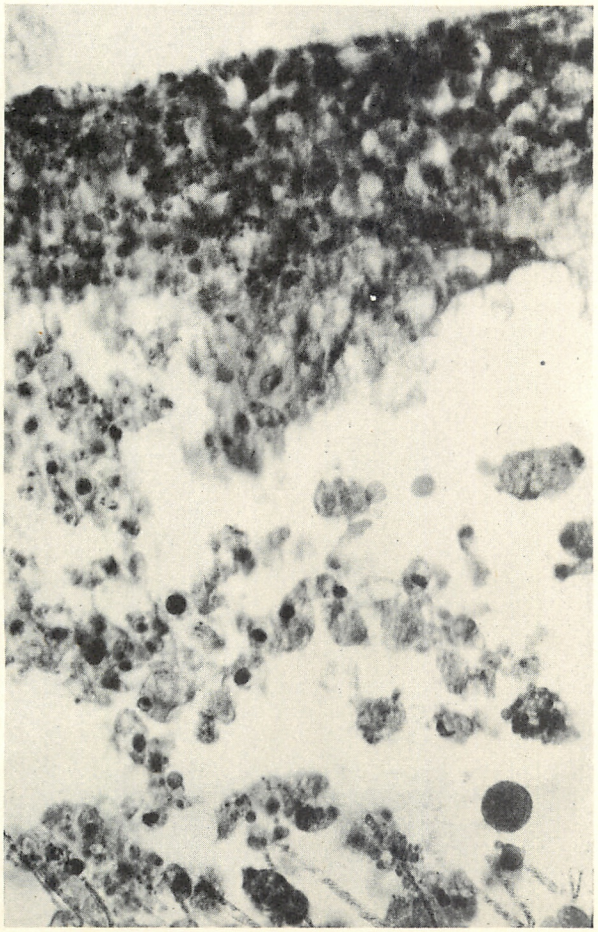
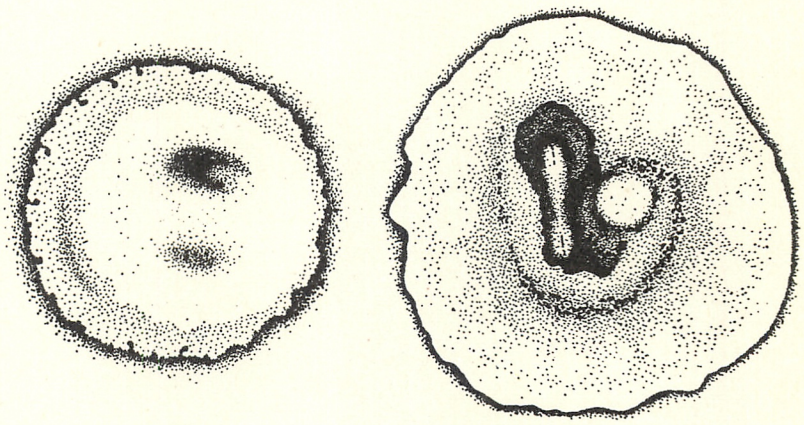


Fig. 16. A segment of the section shown in fig. 15 under a higher magnification. x200.



Figs. 17 and 18. A type of moribund turkey blastoderm which appears to have two organizing fields.

Literature Cited

Barfurth, D. 1895. Versuche über die parthenogenetische Forschung des Hühnereies. Arch. f. Entw. Mech. d. Organismen 2:303-351.

Gowe, R. S. 1950. Techniques for identifying fertile hens' eggs. Poultry Sci. 29:409-413.

Hays, F. A. and C. Nicolaides. 1934. Variability in development of fresh-laid hen eggs. Poultry Sci. 13:74-80.

His, W. 1868. Untersuchungen über die erste Anlage des Wilberthierleibes. Leipzig (cited by Barfurth).

Kosin, I. L. 1944. Macro- and microscopic methods of detecting fertility in unincubated hen's eggs. Poultry Sci. 23:266-269.

_____. 1945. The accuracy of the macroscopic method in identifying fertile unincubated germ discs. Poultry Sci. 24:281-283.

_____. 1948. Parthenogenesis and abortive embryonic development in the domestic fowl. Proc. 8th World's Poultry Congress 1:345-351.

_____. 1949. Moribund and dead blastoderms - factors in the hatchability complex of turkey eggs. Poultry Sci. 28:771 (abstract).

_____. 1951. The prevalence of early embryonic mortality in the Broad Breasted Bronze turkeys. (in press).

Lecaillon, A. 1910. Le parthénogenése chez les oiseaux. Segementation et degenerescence de l'oeuf non féconde. Arch. d'anat. microsc. 12:511-638.

Needham, J. 1942. Biochemistry and Morphogenesis. Cambridge.

Oellacher, J. 1872. Die Veränderungen des unbefruchteten Keimes des Hühnereies im Eileiter und bei Bebrütungsversuchen. Zeit. f wiss. Zool. 22:181-234. (cited by Barfurth).

Romanoff, A. L. 1943. Cultivation of the early chick embryo in vitro. Anat. Record 87:365-368.

Stockard, C. R. 1921. Developmental rate and structural expression. An experimental study of twins, "double monsters" and single deformities, and the interaction among embryonic organs during their origin and development. Amer. J. Anat. 28:115-277.

Taylor, L. W. and C. A. Gunns. 1939. Development of the unincubated chick embyro in relation to hatchability of the egg. Proc. 7th World's Poultry Congress 1:188-190.